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In Silico Evaluation of the Glioma Activity of Reported Compounds from the Extract *Rhodomyrtus tomentosa* (Aiton) Hassk.)

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Abstract: The plant *Rhodomyrtus tomentosa* is empirically treated and can be developed in vitro as an anticancer. To see the interaction and evaluate the compound of *Rhodomyrtus tomentosa* as glioma inhibition, especially on Smoothened receptor by using the in silico. 44 compounds from *Rhodomyrtus tomentosa* leaf plants obtained from previous studies and native ligands and target proteins were generated through PubChem and RSCB protein database. In silico analysis was performed using various, absorption, distribution, toxicity prediction, and molecular tethering of compounds to smoothened (SMO) target proteins. Drug similarity showed that most of the compounds conformed to Lipinski's rule. The absorption and Distribution analysis of the compounds for each parameter gave different pharmacokinetic profiles according to the physicochemical properties of the compounds. Quercetin, β-sitosterol, and Quercetrin are prediction mutagenic, and Rhodomyrtoxin C and β-amyrenonol compounds are What follows is a prediction genotoxic carcinogenic. The results of docking analysis showed that the leaf compounds of *Rhodomyrtus tomentosa* leaf compounds that can interact with SMO receptors with the best interaction shown by compound 13 (Rhodomyrtoxin C) with a free bond energy of -9.29 kcal/mol, Quercitrin of-12.72 kcal/mol, 2-(4-hydroxyphenyl)acetic acid -14.24 kcal/mol and β-Sitosterol of -11,61 kcal/mol and has the same key amino acid residues as the native ligand LY2940680 (4-fluro-N-methyl-N-{1-[4-(1-methyl-1H-pyrazol-5-yl)phthalazine-1yl]piperidin-4-yl} 2 (trifluoromethyl) benzamide)namely Arg400, Asp473 and Glu518. His470, and Asn521. Specific compounds from *Rhodomyrtus tomentosa* are predicted to be developed as candidates for glioma inhibitors predicted to have the same mechanism of action as Smoothened inhibitors and further research is needed.

Keywords: Glioma; Molecular docking; Recepto smoothened; *Rhodomyrtus tomentosa*

Introduction

Cancer is one of the leading causes of death in Indonesia. According to the Global Burden of Cancer Study (Globocan) data from the WHO (2020), there were 19,292,789 cases of cancer in the world, with breast cancer having the highest prevalence of cancer at 2,261,419 (11.7%) cases with a mortality rate of 1,796,144 (18%) cases, which saw an increase from the 2018 data of 11.6% prevalence with a total mortality of 6.6%. Breast

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cancer projections by 2040 are expected to rise to more than 3 million new cases and 1 million deaths each year due to population growth and aging (Arnold et al., 2022).

Cancer is a non-communicable disease caused by the presence of abnormal cells that undergo uncontrolled cell division and the ability of cells to invade other biological tissues, either through direct growth in nearby tissues or by moving cells to distant places (Aisyah et al., 2022; Nur et al., 2021). Cancer biology relies on various signaling pathways in tumor growth and metastasis. One signaling cascade that plays a role in cancer cell growth is the hedgehog signaling pathway (Skoda et al., 2018).

The hedgehog (Hh) signaling pathway is one of the main pathways that control the stages of embryonic development in the form of transmission signals needed for embryonic cells to differentiate appropriately. One of the components involved in the hedgehog signaling pathway is the Smoothened (SMO) receptor. The SMO receptor is an important component of the hedgehog signaling pathway that has an important role in regulating of embryonic development. The Hh signaling pathway begins with binding the Hh ligand to the PTCH 1 receptor and causes the translocation of PTCH 1. Activated SMO then moves towards the cell membrane of the cilia and triggers the activation of the GLI transcription factor by cleaving from the Suppressor of Fused (SUFU) protein thus removing its inhibitory effect. GLI stimulates the transcription of mammalian target genes. Disrupted and aberrant signaling from the Hh pathway can lead to several cancers (Abidi, 2014; Skoda et al., 2018). Not surprisingly, hedgehog signaling is emerging as a target in cancer therapy and several hedgehog inhibitors have been designed. Vismodegib was the first SMO antagonist approved by the FDA in 2012 for metastasis treatment. The drug has some side effects such as muscle spasms, alopecia, and impaired taste. In addition, this drug has been reported to resist SMO receptors. From these weaknesses, it is necessary to develop compounds from natural materials as an approach to cancer treatment through the hedgehog signaling pathway. Modern pharmacological studies have proven that compounds from *Rhodomyrtus tomentosa* exhibit various pharmacological effects including antibacterial, antitumor, anti-inflammatory, and antioxidant. Research conducted by Hamid et al. (2017) that the content of phenolic compounds and flavonoids in *Rhodomyrtus tomentosa* plants has anticancer effects on HepG2, MCF-7, and HT29 cells. Saponin compounds in this plant can have the potential as anti-cancers by inhibiting the formation of Bcl-2. Tannin compounds also inhibit the activity of tyrosine kinase receptors that bind to play a role in the growth of cancer cell malignancy. According to research conducted by Marwati et al. (2020) ethanol extract of *Rhodomyrtus tomentosa* leaves contains total flavonoids of 3.496%, IC_{50} value of 24.451 µg/mL, and has a toxicity effect on shrimp larvae with an IC_{50} value of 31.80 µg/mL in the very strong category An in silico or computational approach is used for research methods using computers or computer simulations. The computational drug design approach is divided into structure-based drug design and ligand-based drug design. These two approaches are complementary and can be adapted to the drug design process (Nur et al., 2023a; Nursamsiar et al., 2020a).

A chemical cannot be productive as a drug if it does not pass through an improper absorption and distribution process that is not maximized by the body. Initial assessment related to pharmacokinetic parameters and toxicity is the main focus for optimizing drug development. This study aims to determine the prediction of absorption, distribution, and toxicity of *Rhodomyrtus tomentosa* leaf isolate compounds. The docking method has three main objectives: predicting the binding of the active side of the ligand, identifying new ligands using virtual screens, and predicting the affinity bond between the compound and the active part of the known ligand. Molecular docking is a major tool in structural molecular biology using computer assistance in designing a drug. Therefore, conducting in silico interaction studies of *Rhodomyrtus tomentosa* leaf compounds against Smoothened (SMO) receptors is necessary. The resulting specific binding interaction provides an overview of target protein inhibition so that it can be used as a reference for further research as a Glioma inhibitor a plant originating from Tana Toraja Regency.

Method

Materials

The hardware used for calculation, molecular modeling, and molecular docking are HP personal computer with Intel ® Core™ i3-10110U CPU @ 2.10GHz 2.59 GHz processor specifications, 4.00 GB memory (RAM) with Windows 11 Home Single Language specifications, ChemOffice 12.0 program package (www.cabridgesoft.com) used to describe the 2D and 3D structures of ligands. HyperChem 8.07 release for Windows (Hypercube Inc.) program was used for geometry optimization using the HyperChem Release v8.07 program. Arguslabv4 0.1 (www.arguslab.com) was used to convert the. a hin file format from Hyperchem 8.07 to the PDB file format. AutoDock 1.5.6 program package was used to prepare the protein structure, ligand structure, grid parameter file, and docking parameter file, while Autogrid 1.5.6 program package was used to prepare the grid,

AutoDock 1.5.6 program package was used to stimulate docking process through Cygwin program package environment, Discover Studio.

Visualizer v2.1.1.0 was used for visualization. The hardware has 2 GB RAM (Random Access Memory) specifications, Intel® Core™ i5 processor, 14" Monitor, 640 GB Harddisk, and Windows® 8 Home Basic 64-bit Operating system. The software used ChemOffice® v.12.0, HyperChem Professional® v.8.00, Marvin® v.21.16, Pre- ADMET® and Toxtree® v.3.1.0 (Nur et al., 2023b; Nursamsiar et al., 2020a).

The materials used are chemical structures in Mol file format (*.mol), 3D ligand binding domain (LBD) data of SMO receptors (PDB ID: 4JKV), and *Rhodomyrtus tomentosa* leaf extract compounds.

Calculation of Physicochemical Values Based on Lipinski's

Rule Physicochemical values were calculated based on Lipinski's rule consisting of molecular weight, log P, hydrogen bond donors, and acceptors using the HyperChem Professional program package® v.8.00 and Marvin® v.21.16 (Nur et al., 2023a).

Parameter Prediction Absorption and Distribution

Absorption and distribution parameters were calculated using the Pre-ADME program® accessed through the website https://preadmet.qsarhub.com/. The compound ligand of the *Rhodomyrtus tomentosa* leaf isolate is uploaded into *mol file format. The program will calculate the predicted value of the selected parameters, namely Human Intestinal Absorption (HIA) parameters, Human colon adenocarcinoma (Caco-2) cells, Mandin Darby canine kidney (MDCK) cells, and Plasma Protein Binding (PPB) (Nur et al., 2023b; Nursamsiar et al., 2020a).

Prediction Toxicity

Toxicity prediction using Toxtree program package® by uploading the compound ligand of *Rhodomyrtus tomentosa* leaf isolate in *mol file format. Toxicity prediction uses the Benigni/Bossa rule-base method to determine carcinogenicity (genotoxic & nongenotoxic) and mutagenicity properties (Nur et al., 2023b; Nursamsiar et al., 2020a).

Preparation of SMO Receptor Molecules

The macromolecule used was the SMO receptor (PDB code: 4JKV) obtained from the PDB database on the website: http/www/pdbbeta.rscb.org/pdb.

Validation of Docking Method

Validation is done by redocking the natural ligand into the active side of the receptor or protein. Docking was performed with default software conditions, with no run or grid changes (Nur et al., 2023b; Nursamsiar et al., 2020a).

Docking Simulation

The structure of SMO receptor (4JKV) and test ligands in the form of 42 compounds of *Rhodomyrtus tomentosa* leaf isolates, and native ligands in *.pdb format were converted into*.pdbqt format through AutoDock Tools 1.5.6 program. The docking method was performed by tethering each ligand to the SMO receptor with Grid Box tethering coordinates (40x40x40) Å. Each ligand is flexible and will interact with AR in a rigid condition. A separate docking process for each grid dimension was performed with the Lamarckian Algorithm in the AutoDock Tools 1.5.6 program package. Evaluation was conducted on the top-ranked conformer from the docking simulation. Evaluation parameters are ligand structure orientation, hydrogen bonds formed, and free energy bond values (Nursamsiar et al., 2020a).

Data Analysis

The obtained data were analyzed at each stage, including the validation of the docking method, the predicted value of Lipinski's rule, the prediction of absorption and distribution, the prediction of toxicity, the chemical interaction of the ligand and the target protein, and the free energy of each ligand. The interaction of the *Rhodomyrtus tomentosa* ligand was compared with that of the native ligand on the target protein.

Result and Discussion

Visualization of All Ligands and Target Proteins

All native ligands and ligands from *Rhodomyrtus tomentosa* were visualized through the Discovery Studio Visualizer v4.5 software package. The visualization results can be seen in Figure 1.

The initial stage in the docking process is preparing ligands and target receptors. There are 2 types of ligands used, namely natural ligands or native ligands and 44 *Rhodomyrtus tomentosa* leaf isolate compounds as test ligands. The native ligand used is LY2940680 (4-fluro-N-methyl-N-{1-[4-[1-methyl-1H-pyrazol-5-yl) phthalazine-1-yl]piperidin-4-yl} 2 (trifluoromethyl) benzamide), In addition to native ligands, test ligands are also used, namely 44 compounds of *Rhodomyrtus tomentosa* leaf isolates, which are drawn in 2D using ChemDraw and then converted to 3D using Chem3D. Conversion to 3D form will help to know the macromolecules that will interact. Then the 3D results will be optimized. Determination of minimal energy is used to minimize energy to obtain the most stable structure for docking with the target receptor (Khotimah et al., 2021; Pratami et al., 2022).

Table 1. Ligand compounds from *Rhodomyrtus tomentosa* plant

Compound structure of *Rhodomyrtus tomentosa* leaf isolate	Molecular Formula	Compound Group
Rhodomyrtone	$C_{26}H_{36}O_6$	Floroglucinol
Rhodomyrtoson A	$C_{26}H_{32}O_7$	Floroglucinol
Rhodomyrtoson B	$C_{26}H_{34}O_6$	Floroglucinol
Rhodomyrtoson C	$C_{41}H_{54}O_8$	Floroglucinol
Rhodomyrtoson D	$C_{25}H_{32}O_6$	Floroglucinol
Rhodomyrtoson G	$C_{26}H_{32}O_7$	Floroglusinol
Rhodomyrtoson H	$C_{26}H_{34}O_6$	Floroglucinol
Rhodomyrtoson I	$C_{28}H_{30}O_6$	Floroglucinol
Rhodomenton A	$C_{25}H_{34}O_7$	Terpenoids
Rhodomenton B	$C_{30}H_{48}O_5$	Terpenoids
Rhodomyrtoxin	$C_{24}H_{28}O_7$	Dibenzofurans
Rhodomyrtoxin B	$C_{24}H_{28}O_7$	Dibenzofurans
Rhodomyrtoxin C	$C_23H_26O_7$	Dibenzofurans
Ψ-Rhodomyrtoxin	$C_{24}H_{28}O_7$	Dibenzofurans
Tomentodion A	$C_{30}H_{46}O_3$	Terpenoids
Tomentodion B	$C_{30}H_{46}O_3$	Terpenoids
Tomentodion E	$C_{30}H_{46}O_3$	Terpenoids
Tomentosanol D	$C_{15}H_{10}O_7$	Terpenoids
Tomentoson A	$C_{41}H_{62}O_9$	Hexacyclic floroglucinol
Myricetin 3-O-α-furanoarabinoside	$C_{23}H_{24}O_{12}$	Flavonoids
Myricetin 3-O-β-D-glucoside	$C_{22}H_{22}O_{12}$	Flavonoids
Myricitrin	$C_{21}H_{20}O_{12}$	Flavonoids
Combretol	$C_{20}H_{20}O_8$	Flavonoids
Quercetin	$C_{17}H_{14}O_7$	Flavonoids
Lupeol	$C_{30}H_{50}O$	Triterpenoids
Betulin	$C_{30}H_{50}O_2$	Triterpenoids
21α H-hop-22(29)-en-3β, 30- diol	$C_{30}H_{50}O_2$	Triterpenoids
3β hydroxy-21α H-hop-22(29)-en 30-al	$C_{30}H_{48}O_2$	Triterpenoids
α-amyrin	$C_{30}H_{50}O$	Triterpenoids
β-amyrenonol	$C_{30}H_{48}O_2$	Triterpenoids
2-(4-hydroxyphenyl)acetic acid	$C_{30}H_{50}O$	Triterpenoids
3β-acetoxy-11α,12α-epoxyoleanan-28, 13β-olide	$C_{32}H_{48}O_5$	Triterpenoids
3β-acetoxy-12-oxo-oleanan-28, 13β-olide	$C_{32}H_{48}O_5$	Triterpenoids
2,3-hexahydroxy diphenyl-D-glucose	$C_{23}H_{24}O_{14}$	Tannins
Tomentosin	$C_{36}H_{28}O_{22}$	Tannins
Casuariin	$C_{42}H_{28}O_{26}$	Tannins
Castalgin	$C_{34}H_{24}O_{21}$	Tannins
Peducalgin	$C_{32}H_{22}O_{20}$	Tannins
Piceatannol 4-O-β-D-glucoside	$C_{20}H_{22}O_9$	Stibenoids
4,8,9,10-tetrahydroxy-2,3,7-trimethoxy-anthracene-6-O-β-D-glucopyranoside	$C_{26}H_{23}O_{13}$	Napthalenoids
2,4,7,8,9,10-hexahydroxy-3-methoxyanthracene-6-O- α-L-rhamnopyranoside	$C_{21}H_{22}O_{12}$	Napthalenoids
β-Sitosterol	$C_{29}H_{50}O$	Steroids
Quarcitrin	$C_{21}H_{20}O_{11}$	Flavonoids

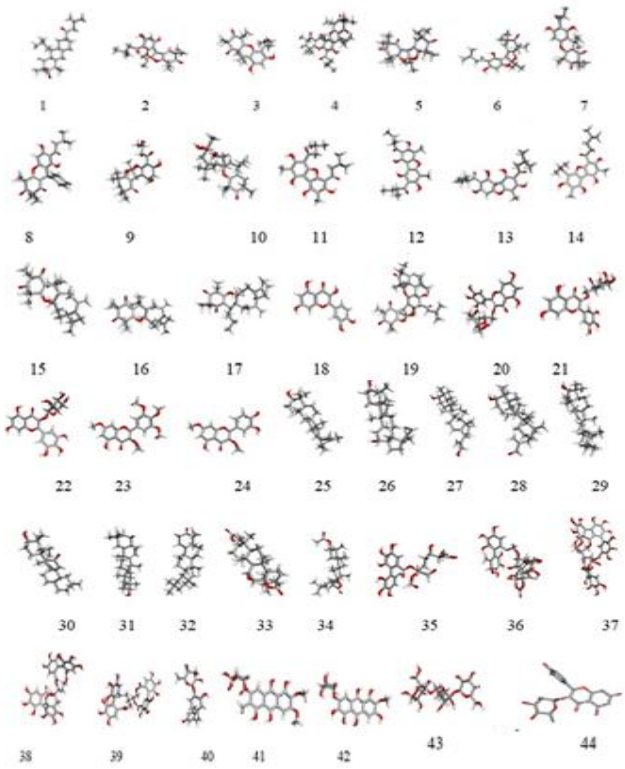

Figure 1. Visualization results of native ligand and *Rhodomyrtus tomentosam* ligand using Discover Studio Visualizer v17.2.0 program package. The names of the ligands are adjusted to the order listed in Table 1 are standard references as anti-glioma drugs

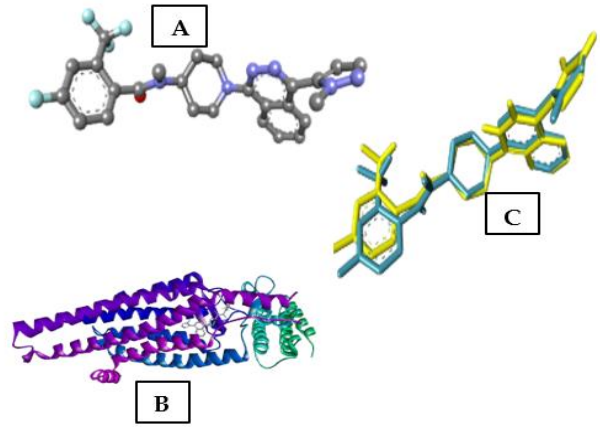

Figure 2. Visualization of target proteins structure of SMO receptorn and native ligand

In this study, the cancer receptor Smoothened (SMO) was used as a target receptor downloaded on the PDB site with the code 4JKV. SMO was chosen because this receptor is a major component in the hedgehog signaling pathway which is very influential in the activation of GLI proteins that play a role in gene transcription. In the last decade, it was reported that several anticancer agents have chemoresistance to this SMO receptors (Amendola et al., 2016).

The downloaded target protein structure is freed from water molecules because it will affect the discovery of the ligand binding position and the calculation of the binding affinity between the target protein and the ligand (Nur et al., 2024, 2022; Wong & Lightstone, 2011). After the removal of water molecules, the addition of hydrogen is also an important concern in molecular docking to obtain a good docking result. In general, the protein structure in PDB contains solvent molecules in the form of water and other residues, so it is necessary to remove water molecules so as not to interfere with the docking simulation process and to ensure the correct interaction between the ligand and the receptor. In addition, it is also necessary to add hydrogen because the presence of hydrogen atoms can affect the results of molecular interactions (Singh et al., 2017; Wong & Lightstone, 2011).

Calculation of Physicochemical Value

Table 2. Screening results of compounds based on Lipinski's rule

Isolate leaf compounds of *Rhodomyrtus tomentosa*	Lipinski Rule				
	Log P	BM	H-bond donor	H-bond Acceptor	Meet conditions/not
Rhodomyrton	4.20	444.568	2	6	Satisfied
Rhodomyrtoson A	9.22	444.568	2	6	≠Satisfied
Rhodomyrtoson B	4.42	426.553	1	5	Satisfied
Rhodomyrtoson C	6.98	674.875	1	8	≠Satisfied
Rhodomyrtoson D	6.29	428.525	0	6	≠Satisfied
Rhodomyrtoson G	3.33	456.535	2	7	Satisfied
Rhodomyrtoson H	3.63	442.552	2	6	Satisfied
Rhodomyrtoson I	3.29	462.97	2	6	Satisfied
Rhodomenton A	2.11	446.54	3	7	Satisfied
Rhodomenton B	6.08	448.71	2	5	≠Satisfied
Rhodomyrtoxin	-1.11	428.48	4	6	Satisfied
Rhodomyrtoxin B	-0.80	414.46	4	6	Satisfied
Rhodomyrtoxin C	-1.04	414.46	4	6	Satisfied
ψ-Rhodomyrtoxin	-1.28	414.46	4	6	Satisfied
Tomentodion A	7.63	454.69	0	3	≠Satisfied
Tomentodion B	7.63	454.69	0	3	≠Satisfied
Tomentodion E	7.19	440.67	0	3	≠Satisfied
Tomentosanol D	-4.01	302.24	5	7	Satisfied
Tomentoson A	10.43	698.94	1	9	≠Satisfied
Tomentoson B	6.91	688.86	1	9	≠Satisfied
Myricetin 3-O-α-furanoarabinoside	-6.13	450.36	8	12	≠Satisfied
Myricetin 3-O-β-D-glucoside	-6.23	466.35	9	13	≠Satisfied
Myricitrin	-5.72	464.38	8	12	≠Satisfied
Combretol	-4.88	388.37	1	8	Satisfied
Quercetin	-4.01	302.24	5	7	Satisfied
Lupeol	8.03	426.73	1	1	≠Satisfied
Betulin	6.12	414.67	2	2	≠Satisfied
Betulin-3-acetate	7.78	500.76	1	3	≠Satisfied
21α H-hop-22(29)-en-3β,30-diol	7.03	442.73	2	2	≠Satisfied
3β hydroxy-21αH-22(29)-en-30-al	6.85	440.71	1	2	≠Satisfied
α-amyrin	7.98	426.73	1	1	≠Satisfied
2-(4-hydroxyphenyl)acetic acid	-14.24	440.71	1	2	Satisfied
Taraxerol	8.09	426.73	1	1	≠Satisfied
Friedelin	8.82	426.73	0	1	≠Satisfied
3β-acetoxy-12-oxo-oleanan-28,13β-olide	6.92	512.73	0	3	≠Satisfied
3β-acetoxy-11α,12α- epoxyoleanan-28, 13β-olide	6.24	512.73	0	3	≠Satisfied
2,3-hexahydroxydiphenyl-D-glucose	-7.08	482.35	9	12	≠Satisfied
Tomentosin	-11.88	784.55	14	18	≠Satisfied
Casuariin	-14.66	934.64	16	21	≠Satisfied
Castalagin	-14.14	936.66	16	21	≠Satisfied
Peduculagin	-10.62	726.51	12	16	≠Satisfied
Piceatannol4-O-β-D-glucoside	-2.83	406.39	7	9	≠Satisfied
β-Sitosterol	4.14	302.04	2	1	Satisfied
Quarcitrin	0.35	414.39	1	2	Satisfied

The development and design of new drugs require various important factors that must be met in achieving a suitable dosage formulation. Increasing solubility is one of the important factors because it can affect the absorption speed and permeability of a drug to produce good bioavailability (Desai et al., 2023). Early predictions related to this need to be made to minimize failure in the development of drug candidates. In this case, computational approaches or "in silico" methods can estimate the initial predictions of a drug candidate before experimental studies are carried out on a laboratory scale. In silico analysis in the design of drug candidates must meet the requirements of the Lipinski rule or "rule of five", namely log P less than 5, molecular weight less than 500, hydrogen bond donors less than 5, and hydrogen bond acceptors less than 10 (Liu et al., 2012; Abla & Banga, 2013; Nur et al., 2023a, 2023b, 2023c; Nursamsiar et al., 2020a).

This study begins with the preparation of compound ligands consisting of 44 compounds of *Rhodomyrtus tomentosa* leaf isolates. Preparation of test ligands is done by drawing the compound structure in the form of 2D and 3D structures, then saved in the Mol file format (*mol). Furthermore, the physicochemical values of the compounds were calculated using the Marvin® and Hyperchem® programs to determine the log P value, molecular weight, hydrogen bond donor, and hydrogen bond acceptor.

The results of screening of *Rhodomyrtus tomentosa* leaf isolate compounds (Table 2) based on Lipinski's rule showed that only 15 compounds met all the criteria of the "rule of five", namely isolate compounds Rhodomyrton, Rhodomyrtoson B, Rhodomyrtoson G, Rhodomyrtoson H, 2-(4-hydroxyphenyl)acetic acid, Rhodomyrtoson I, Rhodomenton A, Rhodomyrtoxin, Rhodomyrtoxin B, Rhodomyrtoxin C, psi-Rhodomyrtoxin, Tomentosanol D, Combretol, Quercetin, β-sitosterol and Quercitrin. Compounds that meet these criteria are predicted to have good absorption or permeation capabilities so that they can be developed into oral preparations.

The compound screening results contained in Table 2 show that only a few compounds of *Rhodomyrtus tomentosa* leaf isolates meet all the parameters or criteria of the "rule of five" that have been set. This shows that the compound does not have a physicochemical profile that is by druglikeness (Lipinski, 2004; Lipinski et al., 2001; Nur et al., 2023a). Compounds that have a molecular weight of more than 500 have poor permeability or permeation. The log P value suitable for oral use is a value of more than one and less than five to have the ability to pass through a good bilayer membrane. Compounds with a log P value of more than five also have the potential to provide toxicity due to low water solubility making it difficult to excrete and accumulate in the body (Gao et al., 2017). Hydrogen bond donor and acceptor values of more than terms also give a poor permeability profile and have an abundant space conformation that will make it difficult to bond with proteins or receptors (Lipinski et al., 2001; Nur et al., 2023a). According to Lipinski et al. (2001), if two parameters or multiple criteria are not met, the system will detect it as a warning of poor absorption and permeability. However, this is not a limitation or prohibition for further modifications and research.

The next step is to determine the values of absorption and distribution parameters. The program used is Pre-ADMET® which is accessed through the website https://preadmet.qsarhub.com/.Pre-ADMET® is a web-based program that can be accessed through a browser. This program calculates molecular descriptors that are closely related to ADME properties based on the Topomol module (plug-in) embedded in the program system. Determination of ADME properties is done by uploading a 2D compound structure in Mol file format (*mol) which will be read by the system and will quickly calculate all 2D descriptors of the uploaded compound (Nursamsiar et al., 2020a).

Based on the screening results of *Rhodomyrtus tomentosa* leaf isolate compounds, 13 compounds meet the "rule of five" requirements. The observation of absorption and distribution parameters of these 15 compounds, showed that only the isolate compounds Rhodomenton A (9), β-sitosterol (43), Quercetrin (44), and Combretol (24) were considered to provide a good pharmacokinetic profile. Other compounds showed fair absorption profile values but had poor distribution profiles based on strong attachment to plasma proteins. The results of the absorption prediction assessment (Table 2) using Human Intestinal Absorption (HIA) parameters showed that almost all compounds had a good ability to be absorbed in the intestine (70-100%). Some compounds that have moderate absorption ability (20-70%) are compounds 18, 25, and 42 and several other compounds that have poor absorption values in the intestine (0-20%) are compounds 21, 22, 23, 37, 38, 39, 40, 41, 43, and 44. The determination of permeability using Caco-2 cell parameters shows that compounds 18, 22, and 25 have low permeability (< 4 nm/sec) and other compounds have moderate permeability (4-70 nm/sec). In the determination of permeability using Mandin Darby canine kidney (MDCK) cell parameters, compounds 5, 9, 18, 25, and 34 have moderate permeability (10-100 nm/sec), and other compounds have low permeability (< 10 nm/sec). In the distribution prediction (Table 2), based on the attachment of compounds to plasma proteins, eight compounds were weakly bound to plasma proteins (< 90%), namely compounds 5, 9, 21, 22, 23, 24, 37, 43, and 44 and other compounds were strongly bound to plasma proteins (> 90%). Compounds that are strongly bound to plasma

proteins are predicted to have poor distribution ability in the body (Nursamsiar et al., 2020b; Adianingsih et al., 2022; Nur et al., 2023a).

Absorption prediction is studied using the Human Intestinal Absorption (HIA) parameter, which is a parameter used to see the percentage of absorption in the human intestine. HIA is the sum of bioavailability and absorption evaluated from the ratio of excretion through urine, bile, and feces. $CaCO_2$ cell parameters are derived from the human colon and have multiple cycles of drug transport through the intestinal epithelium. The permeability coefficient is expressed as the permeability of the $CaCO_2$ monolayer cell culture. Parameter MDCK cells are cells isolated from distal tissue of the canine kidney that differentiate into the columnar epithelium and form tight junctions when cultured on semi-porous membranes. These two in vitro models are used for permeability prediction based on molecular descriptors that explore uptake and efflux transponder mechanisms (Adianingsih et al., 2022; Han et al., 2019; Nur et al., 2023a; Sun et al., 2018). Distribution prediction uses Protein Plasma Binding (PPB) parameters analyzed based on the Quantitative Structure- Activity Relationship (QSAR) which aims to estimate the level of plasma protein binding based on molecular and physicochemical properties of compounds (Han et al., 2019; Sun et al., 2018; Riwu et al., 2022). Based on the assessment results in Table 2, the compounds 2-(4-hydroxyphenyl)acetic acid, Rhodomyrtoson D (5), Rhodomenton A (9), Beta-sitosterol (43), Quercetin (44), and Combretol (24) have the best- predicted values of absorption and distribution parameters compared to other compounds. However, only Rhodomenton A (9), β-sitosterol (43), Quercetin (44), and Combretol (24) compounds meet the Lipinski rule criteria, while Rhodomyrtoson D (5) compounds do not meet some of the criteria set.

The next analysis was the prediction of toxicity properties using the Toxtree® program. This program is software developed by The Joint Research Centre's European Chemicals that is used to estimate or predict structure-based for the determination of several toxicity properties. Toxicity prediction is carried out by entering the 2D compound structure in the Mol file extension (*mol) into the program, then selecting the method used to predict toxicity properties (carcinogenicity and mutagenicity), namely the Benigni/Bossa rule-base method (Benigni et al., 2008).

Polycyclic aromatic hydrocarbons with 3 or more fused rings. The structural warning (nongenotoxic class) contained in the compound structure of *Rhodomyrtus tomentosa* leaf isolate is the o-phenylphenol compound molecular group. The following is a table of *Rhodomyrtus tomentosa* leaf isolate compounds that show carcinogenic potential based on structural warnings in the Toxtree® program.

Table 3. Toxicity prediction

Compounds of *Rhodomyrtus tomentosa* leaf isolates	Carcinogenic		Mutagenic
	Genotoxic	Nongenotoxic	
Rhodomyrton	Negative	Negative	Negative
Rhodomyrtoson A	Positive	Negative	Negative
Rhodomyrtoson B	Positive	Negative	Negative
Rhodomyrtoson C	Positive	Negative	Negative
Rhodomyrtoson D	Positive	Negative	Negative
Rhodomyrtoson G	Positive	Negative	Negative
Rhodomyrtoson H	Positive	Negative	Negative
Rhodomyrtoson I	Positive	Negative	Negative
Rhodomenton A	Positive	Negative	Negative
Rhodomenton B	Positive	Negative	Negative
Rhodomyrtoxin	Positive	Positive	Negative
Rhodomyrtoxin B	Positive	Positive	Negative
Rhodomyrtoxin C	Positive	Positive	Negative
ψ-Rhodomyrtoxin	Positive	Positive	Negative
Tomentodion A	Positive	Negative	Negative
Tomentodion B	Positive	Negative	Negative
Tomentodion E	Positive	Negative	Negative
Tomentosanol D	Negative	Negative	Negative
Tomentoson A	Negative	Negative	Negative
Tomentoson B	Positive	Negative	Negative
Myricetin 3-O-α-furanoarabinoside	Negative	Negative	Negative
Myricetin 3-O-β-D-glucoside	Negative	Negative	Negative
Myricitrin	Negative	Negative	Negative
Combretol	Negative	Negative	Negative
Quercetin	Negative	Negative	Negative
Lupeol2-(4-hydroxyphenyl)acetic acid	Negative	Negative	Negative
β-sitosterol	Negative	Negative	Negative
Quercetrin	Negative	Negative	Negative

Toxicity prediction of *Rhodomyrtus tomentosa* leaf isolate compounds using the Benigni/Bossa rule-base method, analyzing structure-based toxicity estimates of compounds. This method will predict structural alerts to detect carcinogenic and mutagenic properties based on knowledge of the structure embedded in the operating system of the Toxtree® program. Mutagenic activity was predicted based on the *S. typhimurium* TA100 strain developed by McCann & Ames (1976) known as the Ames or The Salmonella test. This in vitro model, consists of a bacterial strain that is sensitive to a large number of DNA-damaging agents. Carcinogenic activity is predicted through the clustering of molecules or substructures associated with the carcinogenic activity of chemicals. Thus, the system can identify the main chemical classes that have the potential to cause cancer (Benigni et al., 2008).

Validation of Docking Method

Validation of the docking method is done by redocking the native ligand on the active side of the SMO receptor. The purpose of this stage is to compare the position of the native ligand to the target protein that has been tested with the position of the copy ligand. The visualization results show that the copy ligand has the same conformation as the natural ligand (Figure 2).

The validation parameter used is looking at the RMSD (Root Mean Square Deviation) value. RMSD value is used to set the similarity of coordinates (pose) between two atoms (Kufareva & Abaygan, 2012). The receptor is declared valid and can continue the docking process of the test compound if it has an RMSD ≤ 2 Å value. Docking validation results between the SMO receptor and native ligand showed an RMSD value of 1.075 Å with the lowest binding energy of -12.30 kcal/mol. These results can be used as parameters in the docking process of test compounds. The grid box size is (40 x 40 x 40) Å with grid box coordinates (x,y,z) -17.004 Å, 6.81 Å, -7.511 Å which will be used to position the grid box to be on the active side of the receptor. These coordinates were chosen because they can cover the amino acid residues that play a role in bonding with the ligand.

Docking Simulation Analysis

The parameters used to determine compounds that have potential as anticancer in this molecular docking are compounds that bind some of the same key amino acids from the interaction between native ligand and SMO receptor (code: 4JKV). The amino acids are Arg400, His470, Asp473, Glu518, and Asn521 (Wang et al., 2013). The next parameter is hydrogen bonding which is the bonding energy between the receptor and ligand. H-bond optimization can optimize the position of hydrogen to donate atoms (both from ligands and proteins). The next parameter observed is binding affinity, which is useful for observing the ability of the drug to bind to the receptor, the smaller the binding affinity value, the more stable the conformation of the compound with the receptor, and the smaller the energy. required to bind. The more negative the free energy value of the bond (ΔG) indicates a good level of stability between the ligand and the target protein (receptor) so that the bond formed will be stronger (Nursamsiar et al., 2020a). The value of free bond energy can be seen in Table 4.

Visualization results show that almost all compounds of *Rhodomyrtus tomentosa* leaf isolates bind to key amino acid residues. Based on the free bond value, the most stable compounds are compounds 25 and 44 with free bond values of -11.00 kcal/mol and -11.22 kcal/mol, respectively. However, both compounds are hydrogen-bonded and do not interact with key amino acid residues on the active side of the receptor.

Table 4. Molecular docking results of *Rhodomyrtus tomentosa* ligand against SMO target protein

Compound	Energy Bond (kcal/mol)
Original ligand (LY2940680 (4- fluro-N-methyl-N-{1-[4-(1- methyl-1H-pyrazol-5-yl)phthalazine-1-yl]piperidin-4- yl}-2(trifluoromethyl)benzamide))	-12.30
Compound 1	-9.83
Compound 2	-3.47
Compound 3	-5.47
Compound 4	+48.23
Compound 5	-3.21
Compound 6	+0.18
Compound 7	-9.78
Compound 8	-6.02
Compound 9	-4.36
Compound 10	+15.40
Compound 11	-7.54
Compound 12	-8.70
Compound 13	-9.29
Compound 14	-8.09
Compound 15	-3.06
Compound 16	-3.67
Compound 17	-3.27
Compound 18	-7.12
Compound 19	+94.14
Compound 20	-4.75
Compound 21	-4.77
Compound 22	-4.27
Compound 23	-7.89
Compound 24	-7.92
Compound 25	-11.00
Compound 26	-9.09
Compound 27	-9.89
Compound 28	-3.03
Compound 29	-9.72
Compound 30	-8.04
Compound 31	-8.52
Compound 32	-11.22
Compound 33	-0.72
Compound 34	-4.47
Compound 35	-2.43
Compound 36	+55.02
Compound 37	+692.67
Compound 38	+105.59
Compound 39	+73.07
Compound 40	-7.47
Compound 41	-8.23
Compound 42	-6.91
Compound 43	-11.72
Compound 44	-11.61

*Bolded letters indicate ligands that best interact with the target protein.

According to Nursamsiar et al. (2020a) the hydrogen bond between the test ligand and the same amino acid residue as the native ligand shows the similarity of the type of interaction in this case describing the similarity of activity. The compound predicted to have the same activity as the native ligand is compound 13 (Rhodomyrtoxin C) which has hydrogen bonds with 3 key amino acid residues namely Arg400, Asp473, and Glu518 with a free energy bond value of -9.29 kcal/mol. This shows that compound 13 is the most stable compound that binds to the SMO receptor. The interaction of the three best compounds and native ligands on the active side of the SMO receptor can be seen in Figure 3.

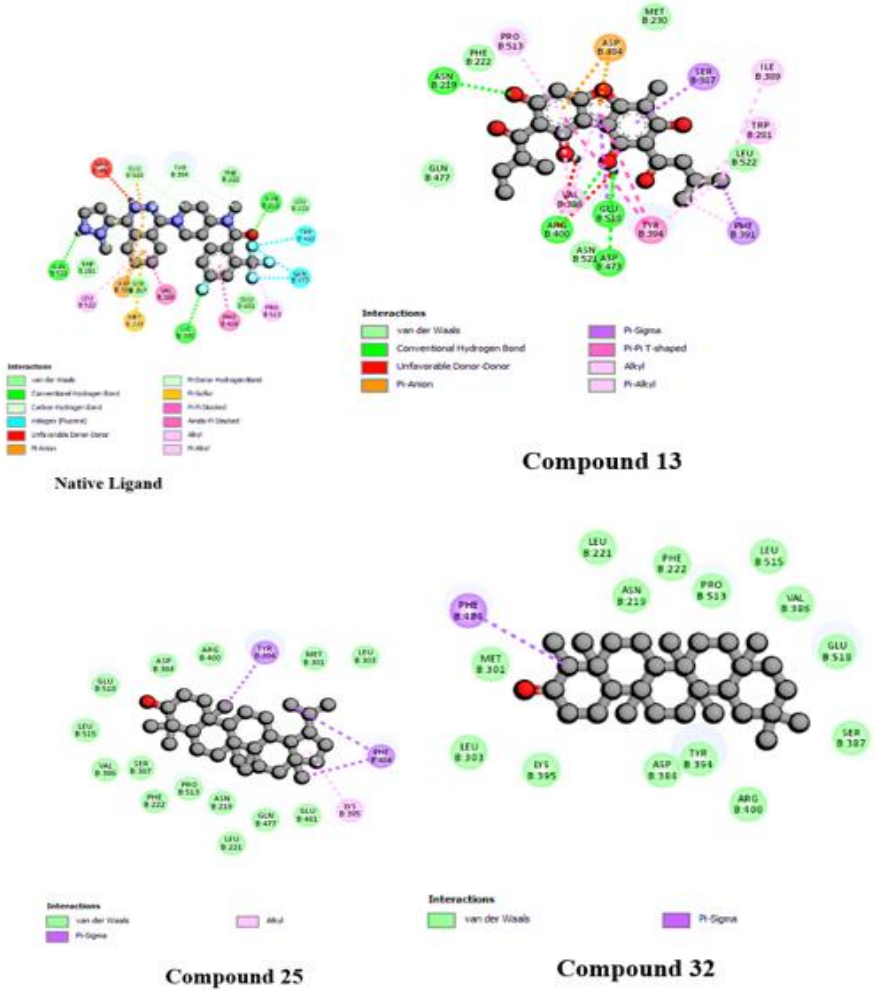

Figure 3. Visualization of molecular docking result between native ligands and the best compounds Form *R. tomenetosa* with SMO target protein

From the docking results between the test ligand in this case, 44 compounds of *Rhodomyrtus tomentosa* leaf isolate. The Smoothened (SMO) receptor, it was also compared with the SMO antagonist drug vismodegib which binds to the same key amino acid residues Asp473 and Glu518 of the SMO receptor: Arg400, His470, and Asn52. However, research reported that vismodegib mutated the key amino acid residue Asp473 in anticancer therapy through SMO receptors (Hiranrat & Mahabusarakam, 2008; Hiranrat et al., 2012a, 2012b; Wang et al., 2013; Hiranrat et al., 2017; Zhao et al., 2020).

Conclusion

Based on the results of research that has been conducted on 44 compounds of *Rhodomyrtus tomentosa* leaf isolates, it shows that the isolate compounds Combretol, 2-(4-hydroxyphenyl)acetic acid, β-Sitosterol and Quarcitrin have the best potential to be further developed as drug candidates. This assessment is based on the suitability of Combretol, 2-(4-hydroxyphenyl)acetic acid , β-Sitosterol and Quarcitrin isolate compounds with drug-likeness, have a fairly good absorption profile on the parameters used, and

good distribution ability because they bind weakly to plasma proteins, and toxicity prediction results show these compounds are neither mutagenic nor carcinogenic and the *Rhodomyrtus tomentosa* leaf isolate compounds tested can be used as drug candidates. *Rhodomyrtus tomentosa* leaf isolate compounds tested can interact with SMO receptors with the best interaction shown by compound 13 (Rhodomyrtoxin C), compound 32 (2-(4-hydroxyphenyl)acetic acid) compound 43 (β-Sitosterol) and compound 44 Quarcitrin with free bond energy of -9.29 kcal/mol, -2.72 kcal/mol, respectively, -11.61 kcal/mol and have the same key amino acid residues as the native ligand LY2940680 (4-fluro-N-methyl-N-{1-[4-(1-methyl-1H-pyrazol-5-yl) phthalazine 1yl]piperidin-4-yl} 2 (trifluoromethyl) benzamide) namely Arg400, Asp473, Asn521, His470 and Glu518. In-silico studies showed that the compound could be developed for further exploration of glioma inhibitor activity.

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Conflicts of Interest
The authors declare no conflict of interest.

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